PCR – using Expand High Fidelity PCR System dNTPack of Roche

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Where

On the bench, PCR machine on the bench left to the door

Material needed

Quantity	What
for 50 μl of PCR mixture:	
10μl	Buffer
0.5μl	of each primer of 100 μM
1 μl	dNTP mix
0.5 μl	Expand High Fidelity Polymerase
0.5 μl	template
– μl	calculate dH_2O quantity needed to have 50μl
	PCR tubes or strips

Steps

1. Use the "Expand High Fidelity PCR System dNTPack of Roche". You can follow the protocol included but it is sufficient to follow the instructions written in this protocol). If you however use another polymerase the corresponding protocol has to be consulted!
2. Add water to primers to make 100 μM
3. Add everything together
4. Put in the PCR thermal cycler (PCR machine), Place carefully and use green plastic rack if you use PCR tubes and not the PCR strips.
5. Program the PCR thermal cycler: 1. 94° C for 2 min,2. 94° C for 20 seconds, 55° C for 30 sec (this depends on the primers), 72° C you have to calculate the time required → the polymerase we use has an e?ciency of 1kbp/min. (E.g. for a piece of 1 kbp we set it to 1min 30sec.) 6. 72° C for 7 min 7. 4° C "forever"
6. Save the file in the MAIN folder → OK→ SAVE→RUN → Select the block you use → RUN → Change sample volume to 50μl, LidT should be 105°C and the box just below ("hotlid") should ALWAYS be checked
7. You can use STATUS to check the progress of the PCR

Warnings

- Sign in for PCR on the White Board just above the machine (write iGEM and from when to when you will use it.)
- It is possible to save polymerase and only do a 10 μl mix if you only want to check if your insert is there and don't actually want to amplify it.